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Origin and Relationship Between Different Cell Types in Malignant Fibrous Histiocytoma

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The derivation of histiocyte-like cells in malignant fibrous histiocytoma (MFH) has been a matter of debate. To shed light on this problem two cell lines from two subsequent recurrencies of MFH were established. The existence of two different cell populations, mainly fibroblast-like in the first cell line and mainly histiocyte-like in the second, was shown by light and electron microscopy, DNA measurements, and karyotype analysis. By detailed banding analysis and identification of several identical chromosomal marker types in the two cell lines, it was proven that they originally derived from the same single cell or single clone. Because the first cell line, with mainly fibroblast-like cells, was in the hypotriploid region and the second, with mainly histiocyte-like cells, was in the penta-hexaploid region, the data explained the appearance of histiocyte-like cells in MFH as a consequence of chromosomal progression. (Am J Pathol 1989, 135:1185–1196)

Malignant fibrous histiocytoma (MFH) is the most common soft tissue sarcoma in adults.[1] Its pathogenesis is largely unknown, although some cases appear secondary to either radiation therapy or pre-existing bone lesions such as Paget's disease, bone infarcts, and aseptic necrosis.[2] MFH-like tumors have also been induced experimentally by, for instance, injection of 9,10-dimethyl-1,2-benzanthracene[3] or SV 40 transformed cells.[4]

Several light and electron microscopic observations were reported, as well as studies on the tumor cells in tissue culture.[5–11] Many of the investigators distinguished two main cell types, ie, histiocyte-like and fibroblast-like, but other less frequent forms like tumor giant cells, undifferentiated cell types, and forms with characteristics of both histiocytes and fibroblasts have also been observed. Histopathology of intact tumor tissue shows a wide range of appearances, and MFHs are currently subclassified into five types according to the predominating morphologic pattern: storiform-pleomorphic, myxoid, giant cell, inflammatory, and angiomatoid.[1] This diversity has led to different views on the histogenesis of, in particular, the histiocyte-like cell type in MFH. Thus, Stout and coworkers[5] proposed a primary histiocytic derivation of MFH, whereas others favored derivation from a primitive stem cell[7] or a dual fibroblastic–histiocytic origin.[11]

The purpose of the present study was to define the origin of and relationship between different cell types present in MFH using observations on two different cell lines established from subsequent recurrencies of one and the same tumor. The techniques employed included light and phase contrast microscopy, electron microscopy, DNA measurements, and chromosome studies.

Materials and Methods

Case Report

In 1977, a 72-year-old man sought medical attention because of a tumor in his left lower arm. The tumor was first noticed by the patient 2 years earlier. The subcutaneous nodule was removed and diagnosed as a malignant fibrous histiocytoma (MFH); it was not considered to be radically excised. An extended resection was performed 2 months later that included skin and the whole extensor carpi radialis longus muscle, as well as the superficial part of the brachioradialis. The defect was covered with a split skin graft. At microscopical investigation, no residual tumor was found. In 1979, however, a recurrence appeared subcutaneously close to the graft. To remove it, three successive excisions were necessary. No tumor was present in the specimen taken from the last excision, and the defect was again covered with split skin.

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In March 1984, a second recurrence was found at the distal margin of the graft. The tumor was localized within muscle tissue and was grossly well circumscribed when removed. However, microscopically there were doubts regarding whether the tumor had been radically excised. From this second recurrence, the first of the two cell lines, U-2149, was established. In June 1984, a third recurrence was observed distally and radially invading the thumb extensor; from this recurrence, the second cell line, U-2197, was established. Because a local excision was incomplete, it was now necessary to perform a total antebrachial amputation. No distant metastases were found at the time of operation, and 1 year later the patient was free from recurrences.

Preparation for Histologic Investigation

Small pieces of fresh tumor tissue, approximately 1 cm in largest diameter, were sampled, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections were prepared and stained according to Weigert/van Gieson. Parallel pieces of fresh tissue were frozen in liquid nitrogen and stored at −70 C for preparation of frozen sections. Both paraffin-embedded and frozen material were used for immunohistochemical staining with the peroxidase-antiperoxidase (PAP) technique.[12]

Cell Lines

Minced fragments of fresh tumor tissue were treated with trypsin and DNase and explanted into 50-mm Petri dishes. Cultures were maintained in Eagle's minimum essential medium (MEM) with 10% Newborn Calf Serum (NCS, Grand Island Biological Company, Paisley, Scotland) and antibiotics (100 units of pencillin and 50 μg of streptomycin per ml). The cells could first be subcultivated after approximately 2 weeks.

Phase-Contrast Microscopy

Cells were seeded sparsely in 35-mm Petri dishes with cover slips. When subconfluent, the cultures on coverslips were fixed for 1 hour at 4 C in 2% glutaraldehyde in 0.15 M sodium cacodylate buffer with 0.1 M sucrose. After washing in PBS, the coverslips were mounted on glass slides and the cells observed in a photomicroscope with phase-contrast equipment.

Light Microscopy of Cell Cultures

For evaluation of cell morphology and counting of different cell types in culture, passages 23 and 60 for line U-2149, and passages 10 and 45 for line U-2197 were used. For comparison, we also studied human foreskin fibroblasts AG 1523 (purchased from the Mutant Cell Repository, Institute for Medical Research, Camden, NJ) in passage 14. Three to 4 days after seeding into 50-mm Petri dishes, cells were washed in PBS, fixed in methanol for 5 minutes, and stained with 10% Giemsa.

Electron Microscopy of Cell Cultures

The cultures were examined in transmission and scanning electron microscopes (TEM and SEM, respectively). For TEM examination, the cells were fixed at 4 C for 24 hours in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer with 0.1 M sucrose. Cultures were rinsed in 0.15 M sodium cacodylate buffer, postfixed in 1% buffered osmium tetroxide for 90 minutes, dehydrated in a series of graded alcohol solutions up to 100%, and embedded in epoxy resin. Sections were contrasted with uranyl acetate and Reynold's lead solution (lead citrate) and viewed in a Philips 201 TEM (Eindhoven, Holland).

Preparation for SEM was the same as for TEM up until the dehydration process, which was performed in acetone solutions. Finally, the cells were dried in Polaron Equipment E 3000 for 1 hour and coated with gold 80%: palladium 20% in Polaron Equipment E 5100 before examination in a Jeol JSM S1 SEM.

DNA Measurement

DNA measurements were made on cultured cells using flow cytometry. U-2149 in passage 63 and U-2197 in passage 45 were seeded onto 100-mm Petri dishes in Eagle's MEM. Cells were harvested after 4 days and prepared for flow cytometry as described by Vindeløv et al.[13] The DNA content was described in relative values, with normal human diploid cells having the value 2c. Trout red blood cells were used as internal standard. For single cell DNA measurement, imprints were made from fresh tumor material and stained according to Feulgen.

Chromosome Preparation and Banding Technique

The chromosomes in different passages of the two cell lines U-2149 and U-2197 were studied with the same G-

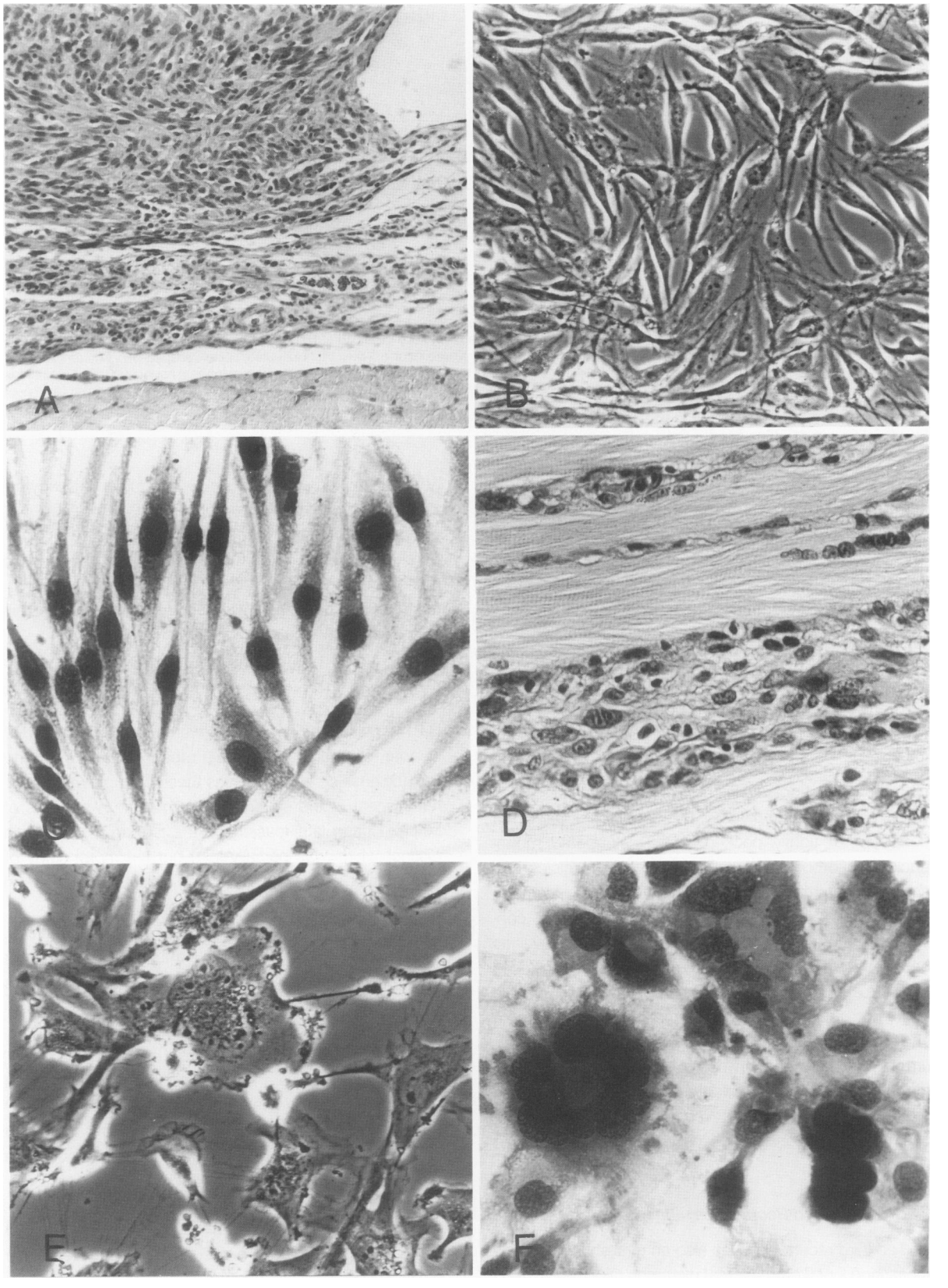

Figure 1. **A–C:** *Second recurrence of MFH, March 1984.* **A:** *Formalin-fixed and paraffin-embedded section stained according to van Gieson (original magnification ×210).* **B:** *Phase-contrast microscopy of corresponding cell line U-2149 passage 42 (original magnification ×250).* **C:** *Giemsa-stained cultures of U-2149 passage 23 (original magnification ×620).* **D–F:** *Third recurrence of MFH, June 1984.* **D:** *Formalin-fixed and paraffin-embedded section stained according to van Gieson (original magnification ×500).* **E:** *Phase contrast microscopy of corresponding cell line U-2197 passage 29 (original magnification ×380).* **F:** *Giemsa-stained cultures of U-2197 passage 10 (original magnification ×620).*

Table 1. *Cell Types in Two MFH Cell Lines*

Cell line	(Passage *in vitro*)	Fibroblast-like cells (%)	Histiocyte-like cells (%)	Multinucleated giant cells (%)	Undifferentiated cells (%)
U-2149	23	67	28	3	1
U-2149	60	64	26	4	5
U-2197	10	19	77	2	3
U-2197	45	19	72	3	5
U-1523	14	96	3	—	2

Percentage of fibroblast-like, histiocyte-like, multi-nucleated, and undifferentiated cell types in two MFH cell lines. Counting was made on Giemsa-stained cultures (Figure 1C, F) under a light microscope (Leitz, Wetzlar, West Germany ×40 objective); 10 × 100 cells were counted in each line. Human foreskin fibroblasts, AG 1523, are shown for comparison. The criteria used were as follows: fibroblast-like cells, elongated, spindle-shaped cells with an elongated nucleus containing one or two nucleoli; histiocyte-like cells, large polygonal or rounded cells, with relatively abundant cytoplasm having ruffled cell membrane with pseudopodia and an oval or reniform nucleus; undifferentiated cells, small, oval or round cells with smooth surface, round nucleus, and a small amount of cytoplasm. Multinucleated cells were specifically noted.

and C-banding techniques previously used for glioma cell lines.[14] The nomenclature follows that of ISCN (An International System for Human Cytogenic Nomenclature, 1985).[15]

Results

Histologic Examination

U-2149 (Second Recurrence, March 1984)

The tumor was localized within muscle tissue and appeared as a grossly well-circumscribed oval-shaped nodule, measuring 1 or 2 cm in cross section. The cut surface was greyish–white and fleshy. Microscopically, the tumor consisted of tightly packed, mainly elongated mononucleated fibroblast-like cells arranged in fascicles and sometimes forming whirls, thus creating a storiform growth pattern (Figure 1A). Each high-power field contained one to a few multinucleated giant cells as well as one to two mitoses. The nodule was diffusely delimited from the surrounding tissue, but neoplastic cells at a distance from the main tumor were confined to fibrous septa and did not invade between individual muscle fibers (Figure 1A). After incubation of frozen sections, but not in formaldehyde-fixed and paraffin-embedded material, the presence of lysozyme and α_1-antitrypsin could be demonstrated immunohistochemically. Staining for desmin was negative.

U-2197 (Third Recurrence, June 1984)

The third recurrence was also an oval-shaped nodule measuring 2 or 3 cm in largest diameter. Like the previous recurrence, it had a greyish-white, fleshy cut surface but was more diffusely demarcated from the surrounding tissues. The microscopic picture was more pleomorphic than was the case with the previous recurrence, with mainly haphazardly arranged plump cells (Figure 1D). Most cells had relatively large, pleomorphic nuclei and abundant cytoplasm, and many resembled histiocytes. Each high-power field now contained four to eight giant cells and four to six mitoses. The tumor lacked sharp borders, and pleomorphic neoplastic cells invaded between muscle cells even at a distance from the main tumor (Figure 1D). Staining for α_1-antitrypsin, lysozyme, and desmin on formalin-fixed material was negative.

Cell Lines

U-2149 cultures (Figure 1B, C) were mainly composed of slender, elongated cells with a broader waist containing the nucleus. In early passages these cells grew in whirls, reminiscent of the storiform pattern seen in sections of MFHs. During later passages, the tendency to grow in distinct whirls was partially lost, but the morphology of individual cells was the same. The cells have so far been maintained in culture up to passage 103.

U-2197 cultures (Figure 1E, F) lacked whirl formations. The cells tended to pile up on one another in a criss–cross pattern. Most were large, flattened, and polygonal cells with extensive lamellae and ruffles and many long filopodia, indicating a high degree of membrane movement. To date, U-2197 has reached passage 45 *in vitro*.

Both cell lines were incubated for the demonstration of a number of enzymes (data not shown). With the PAP technique[12] α_1-antitrypsin was found in moderate amounts in U-2149 and U-2197 and in slight amounts in AG 1523; slight amounts of α_1-antichymotrypsin and lysozyme was noted in U-2149 only. As visualised by cytochemical reactions[16] both U-2149 and U-2197 contained some acid phosphatase and α-naphtylacetate esterase but lacked peroxidase, naphtol AS-D acetate esterase, or naphtol AS-D chloroacetate esterase.

Cell Type Composition of MFH Cell Lines

Because the overall architecture of the two cell lines differed markedly, an effort was made to relate their cellu-

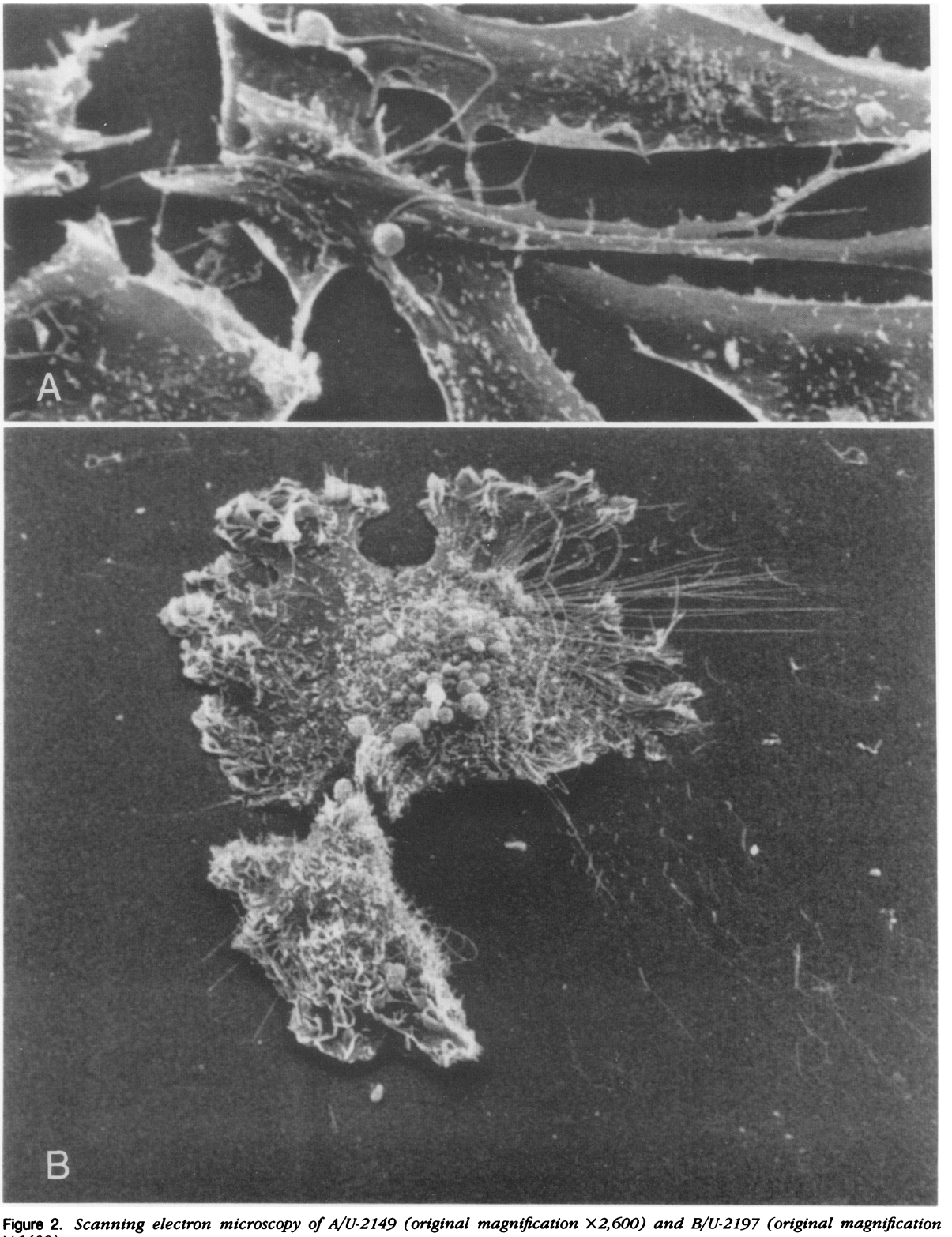

Figure 2. *Scanning electron microscopy of A/U-2149 (original magnification ×2,600) and B/U-2197 (original magnification ×1400).*

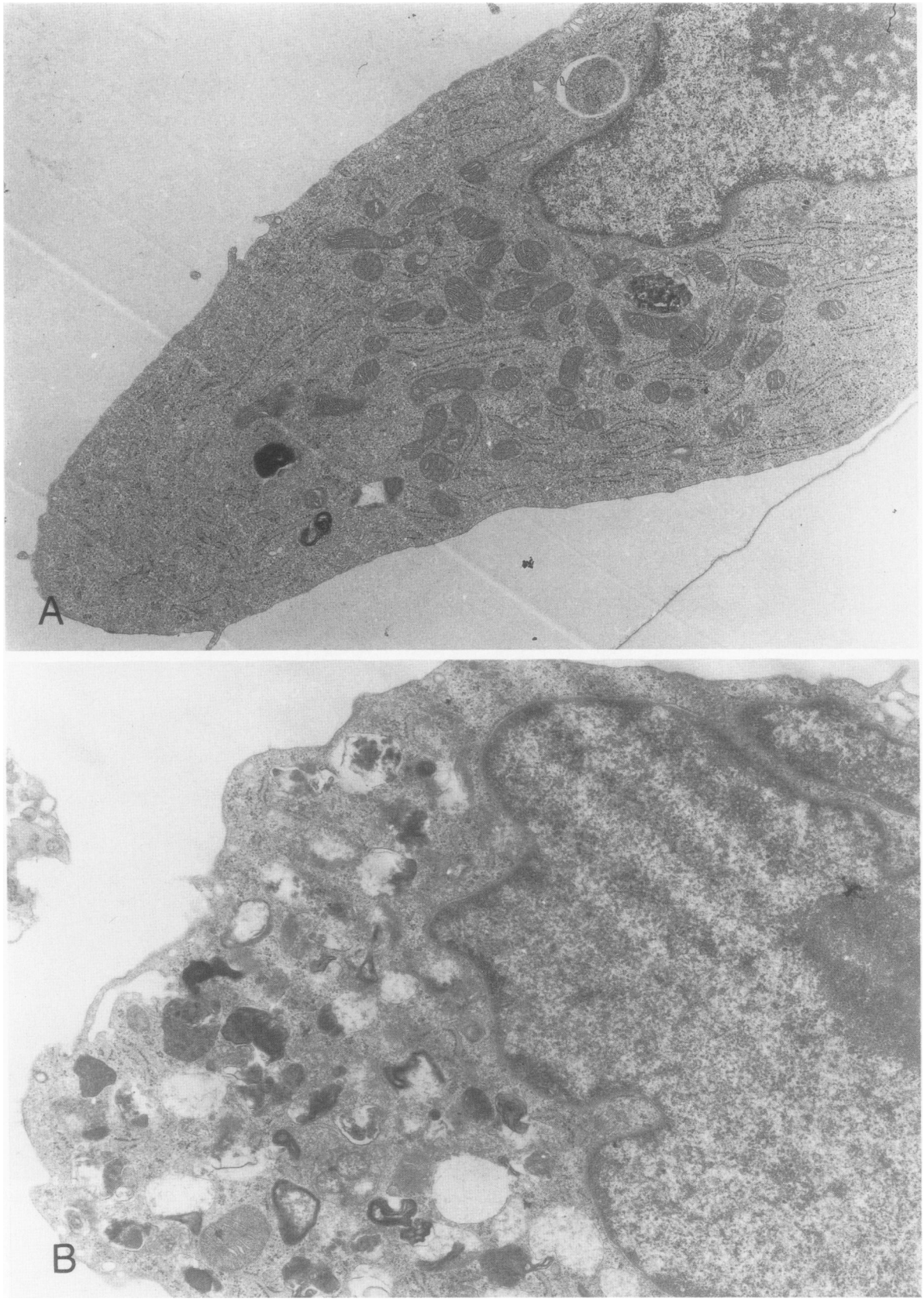

Figure 3. *Transmission electron microscopy of a a fibroblast-like (***A***, original magnification ×15,000) and a histiocyte-like cell (***B***, original magnification ×18,000).*

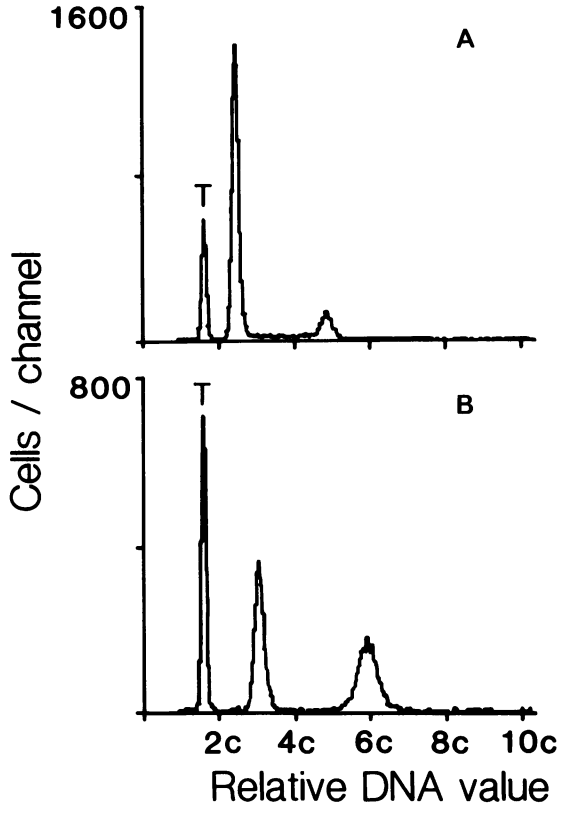

Figure 4. *Relative DNA value in MFH cell lines. Cells were prepared as described by Vindeløv et al*[13] *and measurements were made by flow cytometry with trout red blood cells as an internal standard (T). Relative DNA value of normal human diploid cells was 2c.* **A:** *U-2149 in passage 56 (N = 14.8 × 10³).* **B:** *U-2197 in passage 35 (N = 10.2 × 10³).*

lar composition to earlier reports on the morphology of cell types present in MFH, as described by Fu et al.[7] The percentage of each cell type in the two lines is shown in Table 1 as mean values after counting 10 × 100 cells (see also Figure 1C, F). Although U-2149 contained about 65% fibroblast-like cells, this cell type constituted only approximately 20% of U-2197 cultures. On the contrary, histiocyte-like cells made up the bulk of the U-2197 cultures (approximately 75%). Only a few undifferentiated and multinucleated cells were noted in the two cultures. The cell type composition was approximately the same in the two passages used for each line. For comparison, preparations of diploid human foreskin fibroblasts (AG 1523) were also studied.

Scanning Electron Microscopy

The fibroblast-like cells from U-2149 are shown in Figure 2A as they appeared in the SEM. The cells were elongated and flattened with sparse microspikes or tiny ruffles on their dorsal surface, and thin, small lamellae and small ruffles at the margins. Figure 2B shows the typical appearance of histiocyte-like cells from U-2197. These cells were studded with long, slender microspikes, blebs, and small ruffles. Many cells were supplied with broad lamellae with secondary membrane ruffles, and the presence of retraction fibers was often noted. The migratory path followed by such cells could be imagined from the small fragments of such fibers left behind on the substratum (Figure 2B, lower right).

Transmission Electron Microscopy

Representative examples of the appearance of fibroblast-like and histiocyte-like cells are shown in Figure 3. In both cell lines, fibroblast-like, elongated cells rich in rough endoplasmic reticulum and mitochondria and containing few lysosomes (Figure 3A) were intermingled with histiocyte-like cells with an abundance of lysosomes and vacuoles (Figure 3B). Thus, the two predominant cell types observed by light microscopic examination could also be identified in the electron microscope; they showed the same fine structural characteristics described earlier.[7,9,11] The long filopodia seen in the phase-contrast microscope could be identified in the electron microscope, as could cell types with morphology between fibroblasts and histiocytes.

DNA Measurement

U-2149 showed a major peak, with a relative DNA content of approximately 2.4 c (coefficient of variation [CV] 3.2%) representing 81%, and a minor peak of approximately 4.9 c (CV 3.2%) representing 12% of the cells measured (Figure 4A). Also, in U-2197 two well-defined peaks could be discerned, but the peaks were broader and of higher ploidy. Thus, 46% of the cells had a DNA content of approximately 3.0 c (CV 3.7%), and 48% had a DNA content of approximately 6.0 c (CV 5.0%) (Figure 4B).

Single-cell DNA measurement was made on fresh tumor material giving rise to the U-2149 line. Most of these cells were in the near-diploid range but there was also a fraction of aneuploid cells, most with a DNA content of approximately 2.8 c, and some cells with an even higher relative DNA content (data not shown).

Chromosomal Observations

Table 2 shows the passage number, the chromosome counts and total number of cells studied for each cell line. Recurrent marker chromosomes were found in completely analyzed cells in U-2197 and U-2149. Careful stud-

ies on the presence or absence of such markers resulted in additional partial karyotypes of ten cells of the modal group in U-2197.

In all passages U-2149 had a flat mode in the hypotriploid region. Only a few doubling products of modal cells were seen, and the modal numbers showed a slight tendency to decrease with the increasing number of passages. The karyotypic findings were similar in different passages, and one of the most distinctive features was an extreme variability, with no two cells having an identical karyotype. In spite of this variability, a monoclonal origin of U-2149 cells was obvious from similarities in numerical deviations and from the occurrence of a great number of recurrent identical marker types seen in 30% to 100% of the cells analyzed. The average formula for the modal cells can be written as follows (deviations recorded in relation to the normal diploid karyotype): 63–66, –X, –Y, 1p–, 1q–, 3q–, +3p–q+, 4p–q+, 5p–, 6q–, +6q–, +6q–, 7p+, +7q–, +8, 9p–, +9p–, –10, +11p+, +11p+, 12p+, 12p–, +12p–, –13, +14, +14, +15, –16, 17p+, +17p+, 17p–, –18, +19, +21, +22, +22q+, +22q+, +22q–, +22q–, +5–10 markers of unknown origin. Table 3 shows the origin of the 20 marker types included, which are also illustrated by the partial karyotypes in Figure 5A. The derivation of 15 of these types could be completely clarified and the origin of the remaining five was partially resolved.

Both passages of U-2197 showed a very flat modal distribution in the penta-hexaploid region. This high polyploid modal distribution was derived from doubling products of an original triploid-near-triploid modal cell group that tended to vanish with the increasing number of passages. The karyotypic findings in the two passages studied were similar. Like U-2149, this cell line was characterized by a pronounced chromosomal variability, with all analyzed cells having a unique karyotype. The occurrence of similar structural rearrangements in different modal cells made it clear, however, that they had a monoclonal origin. The average formula for the modal distribution can be written as follows (deviations recorded in relation to a hexaploid karyotype): 105–125, –3X, –3Y, 1q–, –2A1, +2A2, –1–2A3. 3q–, –2–3B4, 2C6q+, 2–4C6q–, +1–2C6q–, 2C7q+, –3C8, 3C9p–, –2C10, 3C11p+, 2–3C12p–, –2–3C12, 2D13p+, –4D13, –3D14, 2D15p+, –3D15, –3–4E16, 4E17p+, 1–2E17p+, +1–2E17p+, –4E18, 2E19q+, –2F20, –2–3G21, 1–2G22q–, 2G22p+q–, +15–35 markers of unknown origin. Table 4 shows the proposed origin of those 18 different marker types whose origins could be wholly or partially clarified, and they are illustrated by partial karyotypes in Figure 5B.

A comparison of Table 3 to Table 4, and of the karyotypes in Figure 5A to those in Figure 5B shows that U-2149 and U-2197 had ten identical marker types in com-

Table 2. *Chromosome Counts in U-2149 and U-2197*

Cell line	Passage number	Chromosome numbers*																	Total cells
		52–57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73–89	100–109	110–119	120–129	130–139	200–300	
U-2149	12 or 13	2^1	1^1	—	—	—	3^3	—	3^3	—	2^1	2^1	3^3	—	—	—	—	1^1	1	2	—	1^1	1	22^{15}
	72	3	1	—	—	1	2	3^1	1^1	3^2	1^1	—	—	—	—	—	—	—	—	—	—	—	—	15^5
	74 or 75	—	1^1	—	4	1^1	3^1	2	7^5	1^1	4^1	1^1	1^1	—	—	—	—	1^1	1	1^1	1	1	—	30^{14}
U-2197	24	—	—	—	—	—	—	—	—	—	1^1	—	2^1	—	3^1	2	1	1	2^1	3^2	4^2	5^2	1^1	25^{11}
	43	—	1	2	—	—	—	—	—	—	—	1	—	1^1	—	—	—	2	5^1	3^1	3^1	4^1	1	23^5

* Superscript figures indicate numbers and distributions of cells karyotyped.

Table 3. *Recurrent Marker Types in Modal Cells of U-2149*

Marker M number	Marker type	Copies per cell	Proposed derivation of the markers
M1	1p−	1	t(1;?)(1qter→1p31::?)
M2	1q−	1	del(1)(q11)
M3	3q−	1 or 2	del(3)(q11)
M4	3p−q+	1	t(;?)(:3p14→3q33::?)
M5	4p−q+	1	t(4;?)(:4p11→4q34-35::?)
M6	5p−	1	del(5)(p11)
M7	6q−	1 or 2	del(6)(q15)
M8	6q−	1 or 2	del(6)(q13)
M9	6q−	1 or 2	i(6p)
M10	7p+	1 or 2	t(7;?)(7qter→7p22::?)
M11	7q−	1	del(7)(q22)
M12	9p−	2	del(9)(p13)
M13	11p+	2	t(5;11)(11qter→11p15::5q12→5qter)
M14	12p+	1	t(12;?)(12qter→12p12::?)
M15	12p−	2	del(12)(p12)
M16	17p+	1-3	t(17;?)(17qter→17p12::?)
M17	17p+	1	i(17q)
M18	17p−	1	del(17)(p11)
M19	22q−	2	del(22)(q13)
M20	22q+	1 or 2	t(22;13)(22pter→22q13::13q14→13qter)

mon. Furthermore, marker M18 in U-2197 obviously represented a marker 20 in U-2149, the latter having been subject to superimposed structural rearrangements. In addition to the described markers, many sporadic types were recognized in the two lines.

Discussion

This article describes the establishment of two cell lines derived from subsequent recurrences of a malignant fibrous histiocytoma of the storiform–pleomorphic subtype. The cellular composition of the two cell lines differed in that the first line, U-2149, contained mainly fibroblast-like cells and the second, U-2197, predominantly histiocyte-like cells.

In the present detailed cytogenetic analysis, both cell lines showed extensive numerical and structural deviations. These differed from those observed in the few previously reported cases.[17,18] A high frequency of metaphases with a variety of chromosomes associated or fused at their telomeres was seen in several of the cases reported by Mandahl et al.[18] In U-2197 there were at least 4 or 5 metaphases showing from two to five of such association figures. Similar pictures were previously observed by us in various types of advanced tumors (unpublished observations and references 14 and 19) and at present we regard this as an unspecific phenomenon.

The derivation of the histiocyte-like cells in MFH has been a matter of debate. Their phagocytic ability does not speak against their derivation from a primitive fibroblast-like cell, because, under certain conditions, fibroblasts can also be avidly phagocytic.[20] Furthermore, some authors recently presented data arguing against the notion that the phagocytic cells are true bone-marrow-derived macrophages. Thus, Wood et al,[21] using techniques for visualizing surface antigens as well as enzymes specifically present in bone-marrow derived monocyte/macrophage cells, found all MFHs studied to be negative for such markers. Most of the tumors, however, contained membrane-associated 5'nucleotidase and alkaline phosphatase. This was taken to indicate that the tumor cells described as histiocyte-like are, in spite of their phagocytic ability, from an origin other than that of true bone-marrow-derived macrophages. The immunohistochemical findings in the present study support this notion. It should be mentioned that some investigators[22] reported that markers for such macrophages can be found in MFHs.

Cytogenetic studies can be highly revealing when the relationship between two cell lines established from one and the same tumor is in question. The existence of two different cell populations in the cell lines described in this work was proven both by DNA measurements and karyotype analysis, and the light and electron microscopic appearance of the two cell types is clearly that of the fibroblast-like and histiocyte-like cells formerly described by several investigators. Detailed banding analyses showed that the two cell lines had no less than ten different types of identical marker chromosomes in common. Several of these markers, eg, 11p+ and 12p−, were unusual and very specific types. In addition, there was an 11th marker type, 22q+ in U-2149, undergoing further structural changes in U-2197 (22p+q−). These structural observations, along with some numerical ones, prove beyond any doubt that U-2149 and U-2197 were derived

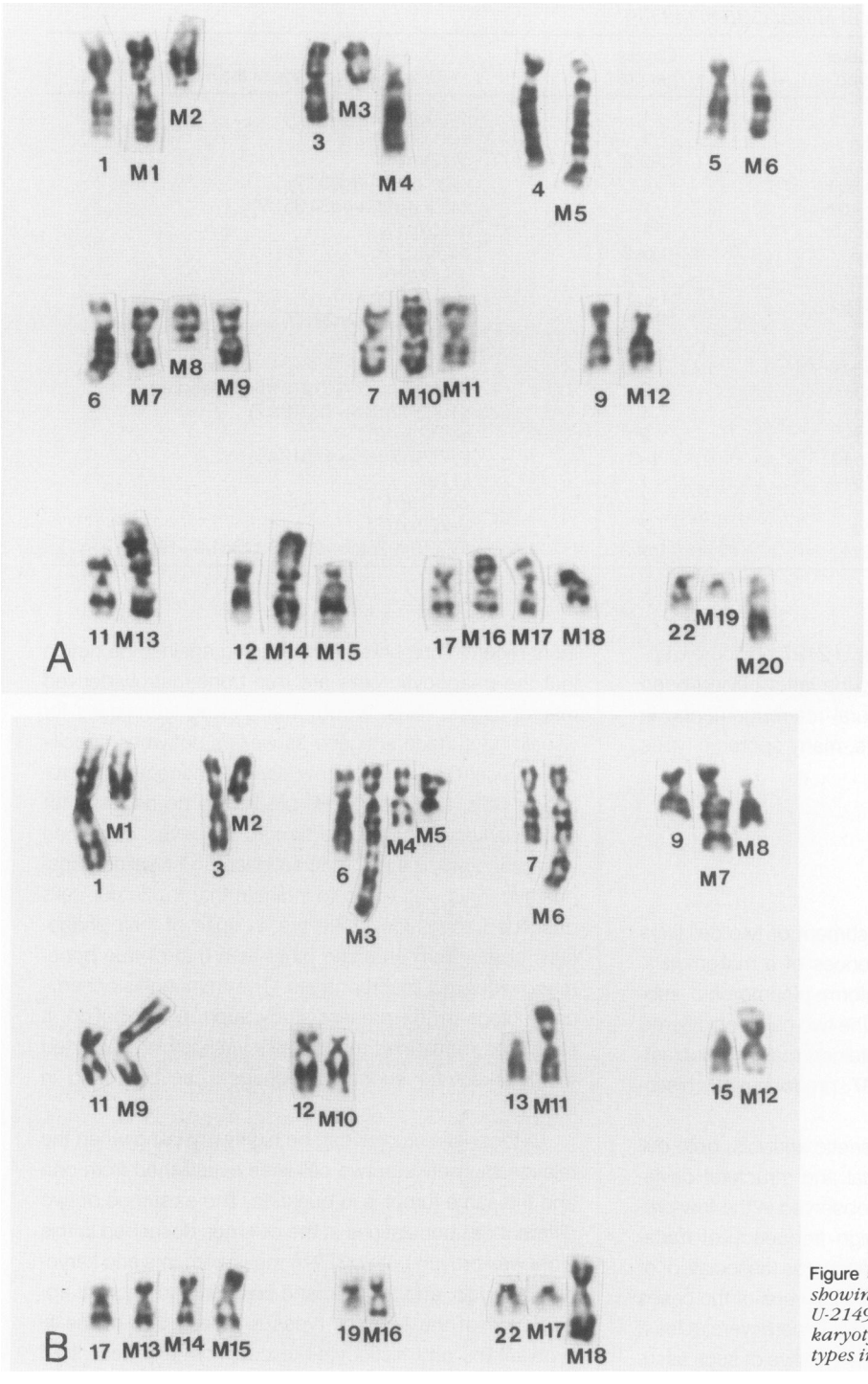

Figure 5. A: *Partial G-banded karyotypes showing the 20 recurrent marker types in U-2149 (see Table 3).* B: *Partial G-banded karyotypes showing the recurrent marker types in U-2197 (see Table 4).*

from the same single cell, or rather single clone. This presumed original neoplastic clone may well have consisted of primitive mesenchymal cells that retained the capacity to differentiate in various directions during progression. The undifferentiated cells present in MFH resemble undifferentiated cells normally occurring in the adventitial region of small blood vessels. Such cells have been regarded as pluripotent mesenchymal cells able to proliferate and take part in tissue repair; theoretically they might give rise to MFHs when transformed.[23]

Our data explain the appearance of histiocytic cells in MFH as a consequence of chromosomal progression. This interpretation fits with a report by Magnusson et al[11] demonstrating that the relative frequency of histiocyte-like

Table 4. *Recurrent Marker Types in Modal Cells of U-2197*

Marker M number	Marker type	Copies per cell	Proposed derivation of the markers
M1	1q−	1	del(1)(q11)
M2	3q−	1 or 2	del(3)(q11)
M3	6q+	4	t(6;?)(6pter→6q13::?)
M4	6q−	4–6	i(6p)
M5	6q−	1–3	del(6)(q15)
M6	7q+	3 or 4	t(7;9)(7pter→7q36::9q11→9qter)
M7	9q+	3 or 4	t(9;9)(9pter→9q34::9q11→9qter)
M8	9p−	2–4	del(9)(p13)
M9	11p+	4–6	t(5;11)(11qter→11p15::5q12→5qter)
M10	12p−	3 or 4	del(12)(p12)
M11	13p+	1 or 2	i(13q)
M12	15p+	3 or 4	i(15q)
M13	17p+	3 or 4	t(17;?)(17qter→17p13::?)
M14	17p+	1 or 2	i(17q)
M15	17p+	1 or 2	t(17;?)(17qter→17p12::?)
M16	19q+	1 or 2	t(19;?)(19pter→19q13::?)
M17	22q−	1 or 2	del(22)(q13)
M18	22p+q+	1 or 2	t(21;22;13)(21pter→21cen::22cen→22q13::13q14→13qter)

cells reflects the degree of malignancy in MFHs in that, the more numerous the histiocyte-like cells were, the more malignant was the tumor. It has been emphasized that the prognosis for patients with MFHs is mainly dependent on tumor size, on the extent of deep growth,[8,24] and on the histologic grade of malignancy.[2] Some investigators also showed a relationship among the prognosis, depth of tumor growth, and cellular pleomorphism.[25] Recently a higher risk of recurrence was reported for aneuploid MFHs than for diploid cases.[26]

The cellular composition of our two cell lines corresponds fairly well with the histologic appearance in the tumorous tissue of origin. In this context, it is notable that the tumor cells giving rise to the second cell line, U-2197, showed a more invasive growth pattern than the cells from the first recurrence, with infiltration occurring between single muscle cells; the corresponding cells in culture, described as histiocyte-like, had a morphology indicating a high degree of membrane movement. The genetic changes responsible for this apparently increased mobility *in vitro* could also be of importance for the higher degree of invasiveness *in vivo*. The present two cell lines, or cloned samples thereof, could be valuable tools for evaluating the multi-step changes taking place during tumor progression.

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